

Microbial DNA Typing by Automated Repetitive-Sequence-Based PCR

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Repetitive sequence-based PCR (rep-PCR) has been recognized as an effective method for bacterial strain typing. Recently, rep-PCR has been commercially adapted to an automated format known as the DiversiLab system to provide a reliable PCR-based typing system for clinical laboratories. We describe the adaptations made to automate rep-PCR and explore the performance and reproducibility of the system as a molecular genotyping tool for bacterial strain typing. The modifications for automation included changes in rep-PCR chemistry and thermal cycling parameters, incorporation of microfluidics-based DNA amplicon fractionation and detection, and Internet-based computer-assisted analysis, reporting, and data storage. The performance and reproducibility of the automated rep-PCR were examined by performing DNA typing and replicate testing with multiple laboratories, personnel, instruments, DNA template concentrations, and culture conditions prior to DNA isolation. Finally, we demonstrated the use of automated rep-PCR for clinical laboratory applications by using isolates from an outbreak of *Neisseria meningitidis* infections. *N. meningitidis* outbreak-related strains were distinguished from other isolates. The DiversiLab system is a highly integrated, convenient, and rapid testing platform that may allow clinical laboratories to realize the potential of microbial DNA typing.

As an epidemiological tool, strain typing is used to assist in tracking the spread of hospital-associated infections (37, 49, 52), food and water contamination (21, 50), and veterinary infections (12). Rapid typing can significantly reduce costs associated with treatment, containment, and decontamination. In addition to the clinical utility of strain typing, there exist multiple applications in both research and industry (1, 5, 8, 29, 52). The food industry uses strain typing for quality control in manufacturing (50). Likewise, strain typing has powerful applications in the fields of biodefense and forensic sciences (30). Due to the multitude of applications, many different techniques have been developed for strain typing (27, 47).

Genotyping methods have increasingly become an integral part of both clinical and research microbiology laboratories. Today, microbial genotyping techniques incorporate molecular biology to reliably distinguish bacterial strains or clones. Genotyping methods include plasmid analysis, restriction endonuclease analysis, PCR assays, multilocus enzyme electrophoresis (MLEE), multilocus sequence typing (MLST), pulsed-field gel electrophoresis (PFGE), DNA sequencing, ribotyping, PCR ribotyping, restriction fragment length polymorphism studies, randomly amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), and repetitive sequence-based PCR (rep-PCR) (40, 41, 42, 46, 47, 54).

Current molecular typing methodologies have limitations (40, 41, 46, 54). Plasmid typing is simple to implement, but

often it cannot discriminate because many bacterial species have either few or no plasmids or maintain similar plasmids (25, 31, 32). MLEE is useful only at providing an estimate of the overall genetic relatedness and diversity (39). Ribotyping (20, 24) and PCR ribotyping (4, 22) often have difficulty distinguishing among different subtypes. Chromosomal restriction fragment length polymorphism and AFLP yield complex DNA profiles that can be challenging to interpret (27, 48). RAPD has high discriminatory power; however, it has poor inter- and intralaboratory reproducibility, due to short random primer sequences and generally low PCR annealing temperatures (27, 47). MLST data are electronically portable, and MLST can be used as a non-culture-based typing method; however, MLST can be labor intensive and costly (10, 33, 40, 54). PFGE is highly discriminatory and is considered the “gold standard” (38); but it has difficulty resolving bands of similar size and there have been issues with interlaboratory reproducibility (7). Essentially, very few typing methods assess outbreaks in real time (33, 54), provide comprehensive surveillance or epidemiological data (6, 36), and have data-archiving capability, all of which are required to build libraries and share data among laboratories (34).

The rep-PCR method uses primers that target noncoding repetitive sequences interspersed throughout the bacterial genome (19, 35, 44) and is an established approach for subspecies classification and strain delineation of bacteria (43, 47). We standardized rep-PCR with quality-controlled reagents in a kit format, automated the detection and analysis by using microfluidics for rapid detection, and digitized the corresponding information in a software package that allows simplistic data

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TABLE 1. Summary of the modifications for automated rep-PCR^a

Rep-PCR condition	Method	
	Manual ^b	DiversiLab
Reaction vol (μl)	50	25
Template (ng)	100	50
Primer(s)	References 19 and 44	References 19 and 44
Buffer	Gitschier ^b plus DMSO and BSA ^c	ABI; no DMSO or BSA ^c
Annealing temp (°C)	38–60	50–70
Cycling parameters	95°C for 7 min, 35 cycles (each cycle, 90°C for 30 s, annealing for 1 min, and 65°C for 8 min), 65°C for 16 min, and 4°C holding	94°C for 2 min, 35 cycles (each cycle, 94°C for 30 s, annealing for 30 s, and 70°C for 1.5 min), 70°C for 3 min, and 4°C holding
Cycle time (h)	6.5	2
Fragment size	50 bp–10 kb	50 bp–4 kb
Amplicon separation and detection	Agarose gel	Microfluidics
Assay setup	1 h	5 min
Total run time	6 h	55 min
Biohazardous waste	Ethidium bromide, UV light exposure	<30 μl of 1.5% acrylamide in chip
Amplicon vol (μl)	10	1
Data capture	High-resolution digital camera; manually	Agilent 2100 bioanalyzer; automatically
Time for 12 samples (h)	8	1
Analysis and reporting	BioNumerics	DiversiLab
Stable algorithm	No	Yes
Interface	Local stand-alone or network	Secure, internet based; unlimited access
Data upload	Manual	Automatic
Sample demographics	Required for each gel	Single entry for each isolate
Report	No	Multiple options

^a This table is intended for general comparison of manual and automated rep-PCR protocols; some conditions may vary depending on source.

^b Reference 44.

^c DMSO, dimethyl sulfoxide; BSA, bovine serum albumin; ABI, Applied Biosystems, Inc.

archiving, retrieval, and reporting. This study was designed to validate the automated rep-PCR method by (i) describing the modifications to manual rep-PCR for automated rep-PCR, (ii) determining performance, including rep-PCR fingerprint pattern stability and chip, user, laboratory, and instrument variability, and (iii) demonstrating applications of the system with *Neisseria meningitidis* isolates. We present the development, performance, and several clinical applications of the DiversiLab system as a compelling tool for clinical laboratories interested in molecular epidemiology.

MATERIALS AND METHODS

Conversion of manual rep-PCR to automated rep-PCR. To increase reproducibility and decrease time and cost, modifications of PCR chemistry and PCR cycling parameters were implemented as summarized in Table 1 (44, 46). Reaction mixture volumes were reduced from 50 to 25 μl, and the quantity of template DNA was reduced from 100 to 50 ng per reaction mixture. The Gitschier buffer was replaced with rep-PCR buffer plus AmpliTaq buffer (Applied Biosystems, Foster City, Calif.). Cycling parameters were modified to increase reaction stringency, decrease amplicon sizes and cycling times, and improve assay reproducibility and ease of detection. Amplicon detection was automated with a microfluidics-based BioAnalyzer (Agilent, Palo Alto, Calif.) and LabChip device, reducing setup time from 1 h to 5 min and decreasing total run time from 6 h to 55 min.

Bacterial isolates. Several bacterial isolates were selected for reproducibility and stability studies. Two human *Escherichia coli* O157:H7 isolates were purchased from the *Escherichia coli* Reference Collection, EHEC1 reference strain set. Strain 93111 is a strain isolated from a human source in 1993, and strain G5101 is a glucuronidase-producing (methyumbelliferyl-β-glucuronide-positive) isolate obtained from a human source in 1995. One vancomycin-resistant *Enterococcus faecium* isolate and five methicillin-resistant *Staphylococcus aureus* (MRSA) isolates were selected for reproducibility studies. A set of 16 *N. meningitidis* isolates were collected by one of us (C.W.) and sent in a blinded fashion for strain typing. Seven of the *N. meningitidis* isolates caused a school-based

outbreak in 1981 (11); an eighth isolate was not associated with an outbreak. Four other *N. meningitidis* clones were clinical isolates originally from Spain (26), and four clones were clinical isolates recently collected in North Carolina. The 12 *N. meningitidis* isolates from Houston and Spain were previously characterized by manual rep-PCR (Table 2) (51). Three ATCC isolates (13102, 13077, and 13090) were used as controls.

Bacterial culture and DNA extraction. All isolates were cultured on trypticase soy agar II with 5% sheep blood, nutrient agar, or nutrient broth for 24 h at 37°C. For the stability studies, both *E. coli* O157:H7 strains were inoculated from frozen stock culture onto trypticase soy agar II containing 5% sheep blood and nutrient plates and incubated at 37°C for 24 h. The original cultures were successively subcultured 10 times over 30 days, stored in the refrigerator for 2 and 4 weeks, frozen to –70°C in glycerol five times, and recultured on blood and nutrient plates and nutrient broth. For all isolates, DNA was extracted using a 1-μl loop of plated culture or 1 ml of broth culture and the UltraClean Microbial DNA isolation kit (Mo Bio Laboratories, Solana Beach, Calif.) following the manufacturer's instructions. All DNA solutions were standardized to a concentration of approximately 25 ng/μl, with the exception of the DNA from one *S. aureus* isolate and one *E. coli* isolate (strain 93111). The designated *S. aureus* and *E. coli* isolates were used in reproducibility studies with variable DNA template concentrations of 12.5, 25, 50, 100, and 250 ng/μl.

Automated rep-PCR DNA fingerprinting. The extracted DNA was amplified using the appropriate DiversiLab DNA Fingerprinting kit: *Escherichia*, *Enterococcus*, *Staphylococcus*, or *Neisseria* (Spectral Genomics, Inc., Houston, Tex.), according to the manufacturer's instructions. Briefly, 50 ng of genomic DNA, the appropriate rep-PCR primer mixture provided in the kits, 2.5 U of AmpliTaq, and 1.5 μl of 10× PCR buffer (Applied Biosystems) were added for a total of 25 μl per reaction mixture. The thermal cycling parameters for all of the kits were similar and were as follows: initial denaturation of 94°C for 2 min; 35 cycles of denaturation at 94°C for 30 s; annealing at 45°C (*Staphylococcus*), 50°C (*Enterococcus*), 55°C (*Escherichia*), or 60°C (*Neisseria*) for 30 s; extension at 70°C for 90 s; and a final extension at 70°C for 3 min.

The DNA amplicons were separated with microfluidics chips (LabChip device; Caliper Technologies, Inc.) and a model B 2100 bioanalyzer (Agilent Technologies, Inc., Palo Alto, Calif.). DNA standard markers (used for normalization of sample runs) and 1 μl of the Chip Kit molecular weight ladder (consisting of

TABLE 2. Clinical *N. meningitidis* isolates and demographic information^a

Sample	Serogroup	Demographic data ^b
A	Y	Winston-Salem 5, 2002
B	Nongroupable (not A–C or Y)	Winston-Salem 6, 2002
C	C/2b:P1.2	Houston 2, 1981 outbreak
D	C	Winston-Salem 7, 2002
E	C/2b:P1.2	Houston 1, 1981 outbreak
F	B/4:p1:15	Spain, R1907, 1985–1986
G	C/2b:P1.2	Houston 8, 1981 outbreak
H	C/2b:P1.2	Houston 7, 1981 outbreak
I	B/4:p1:15	Spain, R1922, 1985–1986
J	C/2b:P1.2	Houston 3, 1981 outbreak
K	B	Winston-Salem 3, 2001
L	C/2b:P1.2	Houston 4, 1981 outbreak
M	C/nontypeable	Houston 5, 1981
N	B/4:p1:15	Spain, R1916, 1985–1986
O	C/nontypeable	Spain, R1909, 1985–1986
P	C/2b:P1.2	Houston 6, 1981 outbreak
Q	A	ATCC 13077
R	B	ATCC 13090
S	C	ATCC 13102

^a *N. meningitidis* isolates include seven isolates from an elementary school outbreak in Houston, Tex. All isolates (except ATCC isolates) were previously characterized by manual rep-PCR and or MLEE (51).
^b Demographic data list source of sample (Winston-Salem, N.C., Houston, Tex., or Spain and isolated number, followed by year(s) of collection, association with an outbreak (if applicable), and strain designations (if available).

concentrations of 5 ng/μl of each 200-, 400-, 600-, 800-, 1,000-, 2,000-, 3,000-, and 4,000-bp DNA marker) were used.

Analysis was performed with the DiversiLab software (version 2.1.66) with the Pearson correlation coefficient to determine distance matrices and the un-weighted-pair group method with arithmetic mean (UPGMA) to create dendrograms. Reports were automatically generated and included the dendrogram, electropherograms, virtual gel images, scatter plots, and selectable demographic fields to aid in interpretation of the data.

rep-PCR fingerprinting and analysis of *Neisseria* isolates with BioNumerics software. For software validation, rep-PCR-amplified products from the *Neisseria* isolates were also analyzed with the BioNumerics software package. Ampli-

cons were separated by 1.5% agarose gel electrophoresis (gels, 25 by 25 cm²) containing ethidium bromide (3 μg/ml in gel and in 1× Tris-acetate-EDTA running buffer) for 6 h at 120 V in a recirculating electrophoresis unit. Digital images of the gels were captured with the AlphaImager 2200 (Alpha Innotech Corp., San Leandro, Calif.). Samples were analyzed with BioNumerics software (Applied Maths, Inc., Austin, Tex.) with the Pearson correlation coefficient for distance matrix and the UPGMA with optimization set at 1% to create dendrograms.

RESULTS

Steps toward automated rep-PCR. Figure 1 diagrams the general process of the automated rep-PCR performed in this study. After DNA extraction, the rep-PCR primers bound multiple repetitive DNA sequences that generated amplicons of different sizes during PCR (Fig. 1A). Amplicons were size fractionated with the microfluidics chip and bioanalyzer, and the rep-PCR fingerprints were analyzed with accompanying computer software (Fig. 1B). Results were displayed in a customizable report format containing electropherograms, scatter plot (data not shown), virtual gel images, and demographic information (Fig. 1C).

To increase reproducibility and decrease time and cost, modifications of the PCR chemistry and PCR cycling parameters were made (44, 46) as summarized in Table 1. Primers were tested and validated with isolates of each genus to increase discriminatory power. To automate amplicon detection, the modified rep-PCR was adapted to a microfluidics instrument, a 2100 bioanalyzer. The sensitivity of the microfluidics detection was improved 50-fold relative to that achieved by agarose gel electrophoresis (data not shown).

Effects of various instruments on reproducibility with a DNA molecular weight ladder. To demonstrate the reproducibility of DNA pattern detection and analysis using automated rep-PCR, six DNA LabChip devices were tested in three separate bioanalyzers (A, B, and C) with the DNA molecular weight ladder provided in the chip kit. A total of 108 sample

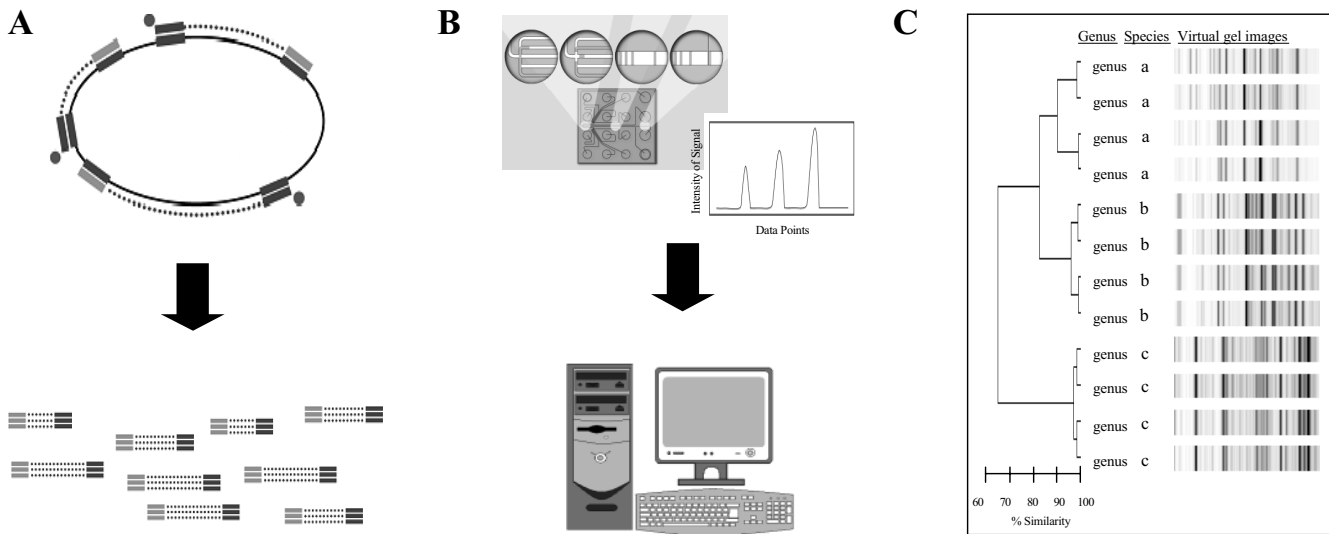


FIG. 1. Schematic diagram of DiversiLab system work flow. (A) rep-PCR primer binds to genomic DNA at multiple sites and allows PCR generation of amplicons of various sizes. (B) The amplicons are separated by microfluidics with the DNA LabChip device, and data are automatically collected and analyzed with the DiversiLab software. (C) A final report is then generated.

wells were compared. The dendrogram and virtual gel images for eight random wells from three different DNA LabChip devices processed on three different analyzers are illustrated in Fig. 2A. The interassay reproducibility exceeded 99%.

Effects of various culture conditions on rep-PCR fingerprint patterns and reproducibility. rep-PCR fingerprint patterns are relatively stable (18, 45). To further evaluate the stability of rep-PCR fingerprint patterns and the reproducibility of those patterns using automated rep-PCR, two different strains of *E. coli* (93111 and G5101) were grown under various culture conditions. A combined total of 64 extractions for both *E. coli* isolates were performed, and the DNA was analyzed by automated rep-PCR. Figure 2B shows the dendrogram and virtual gel images for randomly selected rep-PCR fingerprints obtained from the two subcultured *E. coli* strains, including the primary cultures. Although the *E. coli* isolates were subjected to multiple culture conditions, the rep-PCR fingerprints of each strain appeared stable and reproducible, as indicated by the average similarity (>97.7%) seen in the dendrogram within repeated samples of each strain. The average similarity of the two different *E. coli* strains was 97.2%; however, there were obvious differences between 93111 and G5101 banding patterns, as indicated by the presence or absence of specific PCR amplicons (Fig. 2B). Several other manual extraction methods were also performed, including phenol-chloroform, PrepMan Ultra Sample Preparation reagent (Applied Biosystems), and QIAmp Mini kit (QIAGEN, Inc., Valencia, Calif.). Only the extractions performed with the QIAmp Mini kit gave fingerprint patterns similar to those obtained by the Ultra-Clean method (data not shown).

Effects of DNA template concentration on rep-PCR fingerprint patterns and reproducibility. To examine the effects of genomic DNA template concentration on rep-PCR fingerprint pattern reproducibility, the DNA from the *E. coli* and the *S. aureus* isolates was analyzed by automated rep-PCR. The amounts of template genomic DNA per reaction mixture ranged from 25 to 500 ng, and the rep-PCR DNA profiles did not change appreciably with 10-fold or greater differences in template DNA concentrations. The average similarity values exceeded 98% across the range of template DNA concentrations (Fig. 3A).

Effects of various laboratories, equipment, and personnel on rep-PCR fingerprint patterns and reproducibility. To determine technical reproducibility among laboratory personnel, three different technicians in one laboratory separately performed extraction, rep-PCR, and analysis using automated rep-PCR in triplicate with one isolate each of *E. coli*, *E. faecium*, and *S. aureus*. A total of 81 rep-PCRs were performed. The dendrogram of a random group is shown in Fig. 3B. The rep-PCR fingerprint patterns were virtually indistinguishable within each isolate tested, and the average similarity of all fingerprints with each isolate was >98%. In addition, 12 separate laboratories tested five MRSA isolates in duplicate, for a total of 120 reactions, using the DiversiLab *Staphylococcus* kit. A total of five different thermal cycler models were used: Mastercycler (Eppendorf AG, Hamburg, Germany), iCycler Thermal cycler (Bio-Rad Laboratories, Hercules, Calif.), GeneAmp PCR System 9700 (Applied Biosystems), GeneAmp PCR System 9600 (Applied Biosystems), and PTC-100 cycler (MJ Research, Waltham, Mass.). All operators were first-time us-

ers. The results showed five different rep-PCR fingerprint patterns, and the patterns were virtually indistinguishable within each of the five isolates tested. The average similarity of the replicate fingerprints of each isolate was >96% (data not shown).

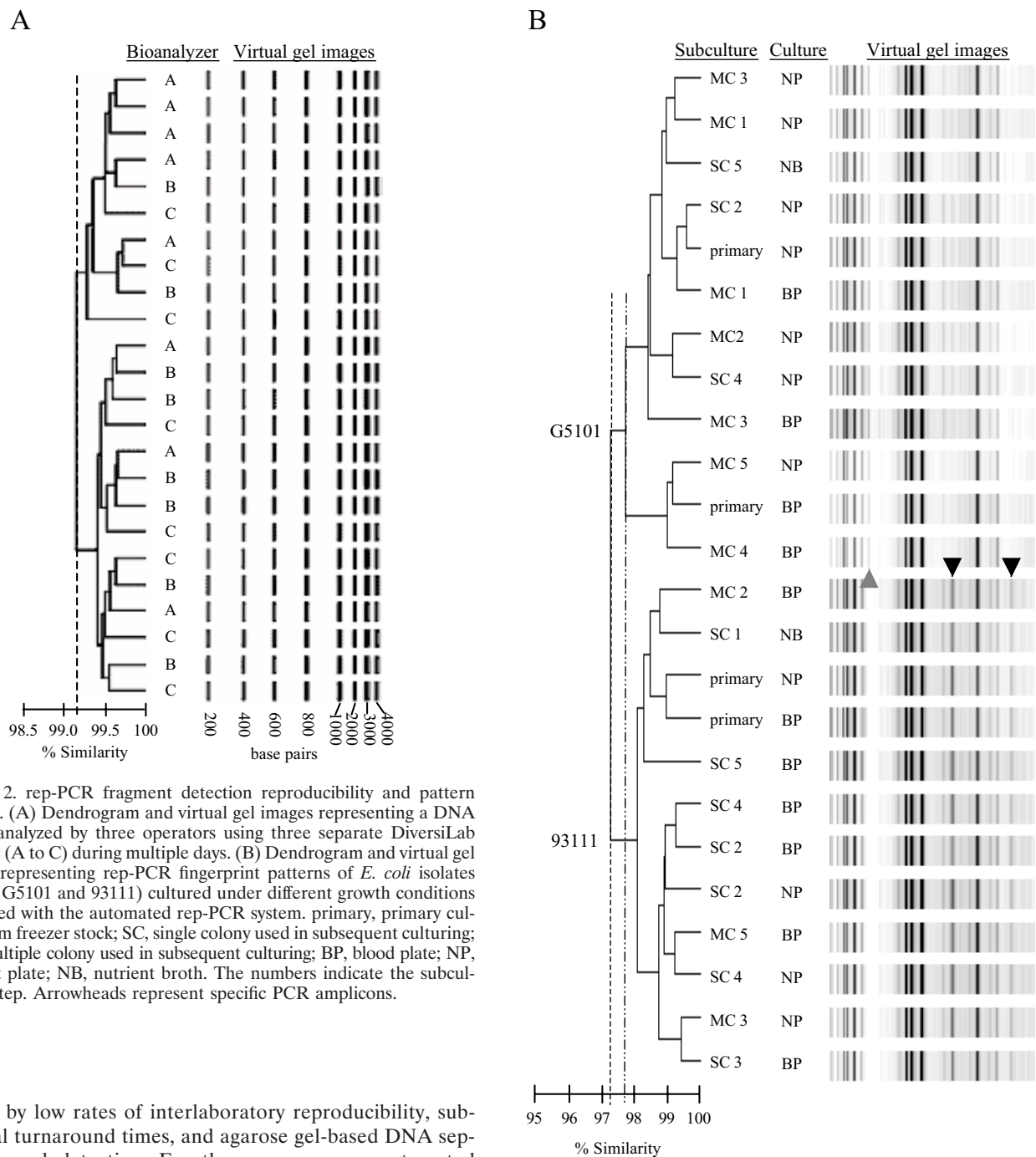
Analysis of *Neisseria* isolates with BioNumerics software. The use of manual rep-PCR and analysis with the BioNumerics software as a tool for source tracking has been published previously (43, 47). To validate the DiversiLab software, the 16 *N. meningitidis* strain DNA profiles were detected by agarose gel electrophoresis and analyzed with the BioNumerics software as a standard for comparison with the DiversiLab analysis. Figure 4A shows the dendrogram and virtual gel image from the BioNumerics analysis. Although the similarity percentages between the BioNumerics and the DiversiLab (Fig. 4B) analysis were not identical (optimization parameters could be modified in BioNumerics but not in DiversiLab), the clustering patterns were similar. Several isolates were only analyzed by automated rep-PCR (Fig. 4B). The numeric data obtained from the BioNumerics-based analyses of the agarose gels was also exported to the DiversiLab software for comparative analyses and validation. Again, the clustering patterns between BioNumerics- and DiversiLab-based dendrograms were consistent (data not shown).

Analysis of *N. meningitidis* isolates by automated rep-PCR. Isolates were analyzed with the DiversiLab *Neisseria* kit (Fig. 4B). The dendrogram and virtual gel images indicate strain-level grouping of the 16 *N. meningitidis* isolates and the three ATCC isolates. The seven isolates collected from the *N. meningitidis* outbreak (E, C, H, G, P, L, and J) clustered together. The sample that was not gathered during an outbreak (M) clustered separately from the other seven isolates. Although all eight isolates were serogroup C, the rep-PCR fingerprint pattern for M was not similar to those in the outbreak or to any other serogroup isolates tested. Isolates N, I, and F (all serogroup B) clustered together as expected, because these isolates were previously typed as a single clone by MLEE. Isolates O (Spain) and D (Winston-Salem), both serogroup C, grouped together. Clinical isolates K (serogroup B) and A (serogroup Y), and isolate B (nontypeable) and ATCC isolates Q (serogroup A), R (serogroup B), and S (serogroup C) clustered separately from all other isolates. As shown by the scatter plot in Fig. 4C, meningococcal isolates yielded serogroup- and strain-level discrimination.

DISCUSSION

This automated rep-PCR-based pathogen DNA-typing platform represents an effective platform for molecular epidemiology in clinical laboratories. The translation of manual to automated rep-PCR systems has resulted in a more convenient, user-friendly, and integrated platform for future developments. Consistent and reproducible DNA profiles were generated with multiple laboratories, personnel, laboratory equipment, various template DNA concentrations, multiple microfluidics instruments, and different culture conditions. Outbreak-related isolates of *N. meningitidis* belonging to serogroup C clustered together and were distinguished from other serogroup C and serogroup B and Y isolates by automated rep-PCR DNA typing.

Manual rep-PCR is useful for strain typing, but it is chal-



lenged by low rates of interlaboratory reproducibility, sub-optimal turnaround times, and agarose gel-based DNA separation and detection. For these reasons, we automated rep-PCR-based typing. Previous studies reported improved reproducibility and discriminatory power with modifications in rep-PCR (27). The combined modifications applied to automate rep-PCR increased reproducibility and decreased turn-around time, cost, and template requirements. One study reported that rep-PCR amplicons separated by agarose gel electrophoresis yielded inconsistent interlaboratory results and was cumbersome in clinical laboratories (16). Adoption of the microfluidics detection platform increased reproducibility by minimizing interprofile variability, reducing fractionation time from 6 to 1 h and decreasing technical time and labor costs. Visual analysis and interpretation of DNA profiles in agarose

gels is time consuming and relatively subjective (34). Software programs may facilitate image analyses, but images must be captured and imported prior to analysis and subjectivity remains because optimization parameters can be modified (34). The DiversiLab software uses a standardized algorithm, and automation allows sample analysis, including a report, to be completed for 13 samples in approximately 4 h, expediting molecular epidemiological studies.

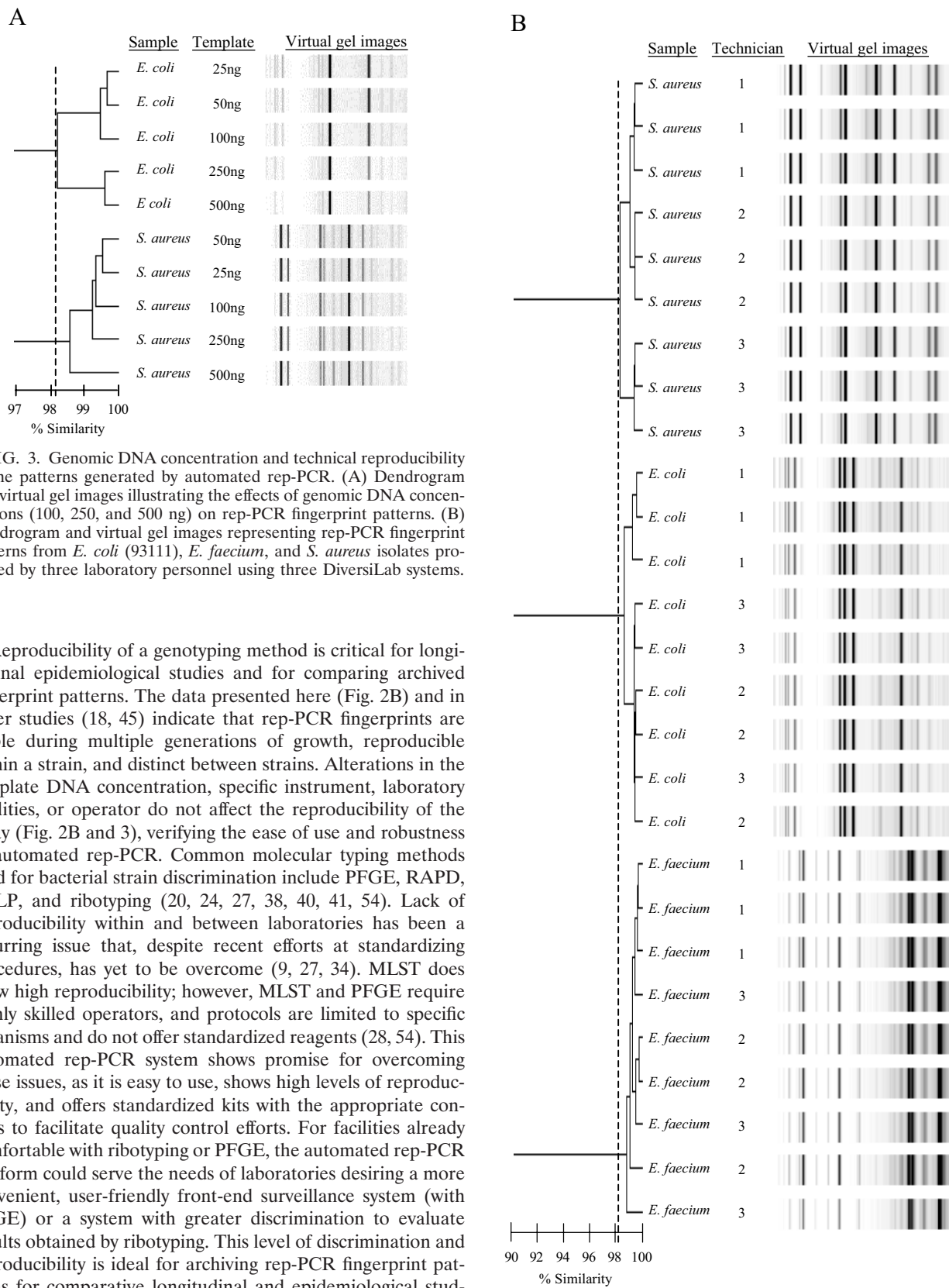


FIG. 3. Genomic DNA concentration and technical reproducibility of the patterns generated by automated rep-PCR. (A) Dendrogram and virtual gel images illustrating the effects of genomic DNA concentrations (100, 250, and 500 ng) on rep-PCR fingerprint patterns. (B) Dendrogram and virtual gel images representing rep-PCR fingerprint patterns from *E. coli* (93111), *E. faecium*, and *S. aureus* isolates processed by three laboratory personnel using three DiversiLab systems.

Reproducibility of a genotyping method is critical for longitudinal epidemiological studies and for comparing archived fingerprint patterns. The data presented here (Fig. 2B) and in other studies (18, 45) indicate that rep-PCR fingerprints are stable during multiple generations of growth, reproducible within a strain, and distinct between strains. Alterations in the template DNA concentration, specific instrument, laboratory facilities, or operator do not affect the reproducibility of the assay (Fig. 2B and 3), verifying the ease of use and robustness of automated rep-PCR. Common molecular typing methods used for bacterial strain discrimination include PFGE, RAPD, AFLP, and ribotyping (20, 24, 27, 38, 40, 41, 54). Lack of reproducibility within and between laboratories has been a recurring issue that, despite recent efforts at standardizing procedures, has yet to be overcome (9, 27, 34). MLST does show high reproducibility; however, MLST and PFGE require highly skilled operators, and protocols are limited to specific organisms and do not offer standardized reagents (28, 54). This automated rep-PCR system shows promise for overcoming these issues, as it is easy to use, shows high levels of reproducibility, and offers standardized kits with the appropriate controls to facilitate quality control efforts. For facilities already comfortable with ribotyping or PFGE, the automated rep-PCR platform could serve the needs of laboratories desiring a more convenient, user-friendly front-end surveillance system (with PFGE) or a system with greater discrimination to evaluate results obtained by ribotyping. This level of discrimination and reproducibility is ideal for archiving rep-PCR fingerprint patterns for comparative longitudinal and epidemiological studies.

Laboratories are taking advantage of advances in information technology, including the use of public electronic strain

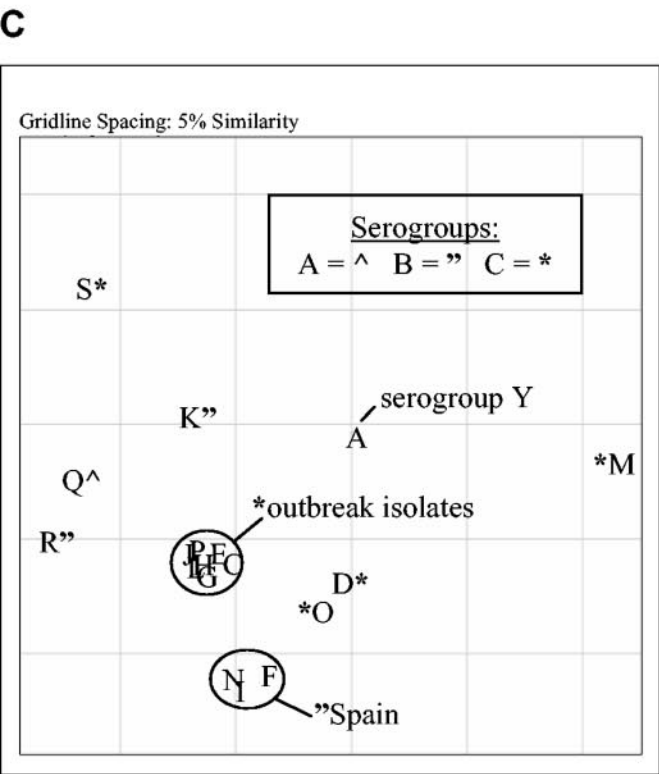
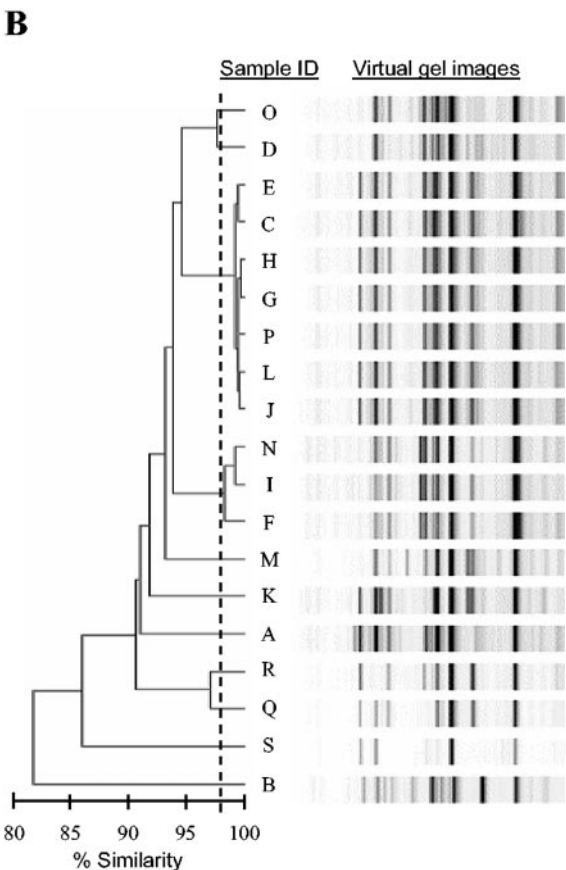
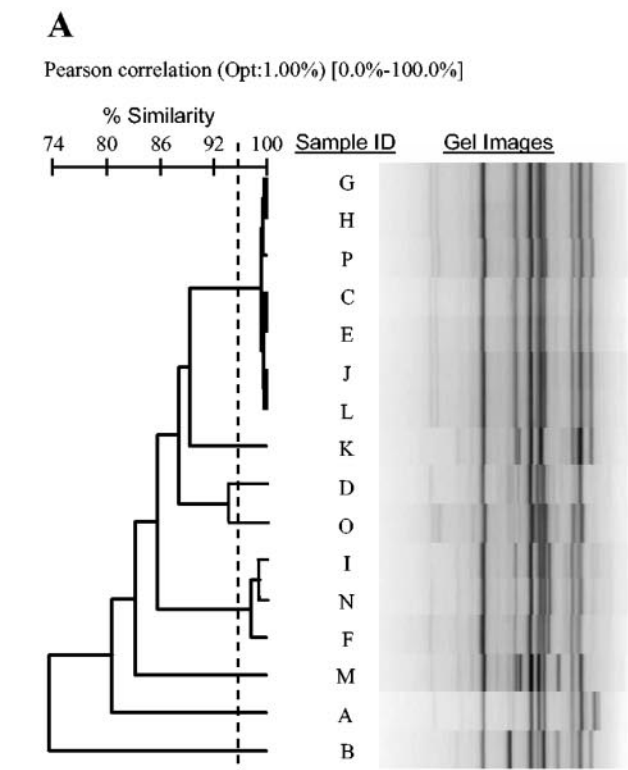


FIG. 4. DNA fingerprint analysis of rep-PCR with BioNumerics and DiversiLab software of *N. meningitidis* isolates. (A) Dendrogram analysis and virtual gel image of rep-PCR fingerprint patterns of *N. meningitidis* isolates (samples A to P) with BioNumerics. (B) Dendrogram analysis and virtual gel images of rep-PCR fingerprint patterns of the *N. meningitidis* isolates (samples A to S) with DiversiLab software, version 2.1.66. (C) Scatter plot of the *N. meningitidis* isolates (samples A to S) with the DiversiLab software, version 2.1.66. Isolates C, E, G, H, J, L, and P were obtained from a single outbreak of disease.

typing databases such as MLSTdbNet and PulseNet of the Centers for Disease Control and Prevention. The DiversiLab software uses a similar format; however, the data transferred from the bioanalyzer to an individual customer website are automated, password protected, and encrypted for security. The web-based formats allow access from any computer connected to the Internet; isolates can be compared to each other or to those contained in databases. Demographic information accompanying a sample must be entered every time a gel is analyzed with BioNumerics, whereas the DiversiLab software only requires a single entry that is linked for archiving results and future analyses of interest to infection control studies.

The trend for strain-typing methods used to support infection control is becoming more organism specific. Several methods have been or are in the process of being validated as gold standards, based on specific genus and/or species. For example, PFGE has generally been considered the gold standard for strain-typing bacteria and remains so for many bacteria such as MRSA. Many strain-typing methods are used for meningococcal isolates, including PFGE, RAPD, fluorescent AFLP, and MLEE (13, 40, 53); yet MLST, which has a public *Neisseria*

database for interpretation, MLSTdbNet (17), has become the gold standard. The results study of *N. meningitidis* (Fig. 4) yielded results with automated rep-PCR that were equivalent to those obtained by manual rep-PCR and in concordance with previous MLEE results (51). The results also showed multiple fingerprint patterns within a serotype, which may be an indication of strain-level discrimination for rep-PCR. Although the sample size was limited, the sample set included a single clonal outbreak group and serotypes from multiple geographic regions. In addition, the set demonstrated the utility of an automated rep-PCR application. The performance validation reported in this study provides the basic information, so further studies with direct comparisons can be completed to validate the use of the automated rep-PCR system for specific organisms. Multiple studies have demonstrated strain-level discrimination for automated rep-PCR with the DiversiLab system, including mycobacteria (2) and fungi of both *Aspergillus* (15) and *Candida* spp. (3, 23).

The medical and economic benefit of a highly integrated, comprehensive infection control program that includes routine genotyping has been previously demonstrated (14). The DiversiLab system has the potential to be the standardized platform for routine bacterial and fungal genotyping in the clinical laboratory. The DiversiLab system offers efficiency, excellent discriminatory power, and reproducibility to allow database building, interlaboratory comparisons, and high throughput applications. The small footprint of the automated system conserves work space, while the web-based interface enables remote access and data sharing for the assessment of population profiles within and among institutions. Finally, rep-PCR-based molecular typing is considered to have favorable costs (particularly startup and equipment costs) associated with this platform, compared to other methods (2, 10, 13, 27, 41). Additional cost savings may be realized by reductions in technologist time, training, or labor. Further automation is possible by coupling automated DNA extraction systems with rep-PCR. In conclusion, our study demonstrates the potential utility of automated rep-PCR and the DiversiLab system as tools for strain tracking in routine pathogen surveillance and disease outbreak investigations.

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